Semi-Annual Status Report

Grant Number:	**NASA-NAG 2-819**
Grant Period:	**1/01/93 - 12/31/95**
Principal Investigator:	**Andrew O. Martinez, Ph.D.**
Institution:	**University of Texas at San Antonio**
Project Title:	**Monoclonal Antibodies Directed Against Surface Molecules of Multicell Spheroids**
Report Period:	**7/01/93 - 13/31/93** 12

Narrative:

The objective of this project is to generate a library of monoclonal antibodies (MAbs) to surface molecules of mammalian tumor and transformed cells grown as multicell spheroids (MCS). These MCS are highly organized, 3-dimensional multicellular structures which exhibit many characteristics of *in vivo* organized tissues not found in conventional monolayer or suspension culture; therefore, MCS make better *in vitro* model systems to study the interactions of mammalian cells. Additionally, they provide a functional assay for surface adhesion molecules.

The aims for this reporting period were: (1) to continue generating a library of hybridomas producing antibodies against surface molecules of human and rodent tumor and transformed cells grown as MCS using a subtractive immunization scheme; and (2) to begin to characterize the binding patterns of selected MAbs from our library on panels of human and rodent cell lines by immunofluorescence microscopy and flow cytometry. To this end, six additional hybridomas producing antibody of interest were identified and added to the library during the reporting period. In addition, five hybridomas which were previously reported were cloned and subcloned to ensure that they were producing monospecific antibody. We also began to characterize the binding patterns of selected MAbs on panels of normal, tumor and transformed human and rodent cell lines (see Appendix for list).

Preliminary results of these studies have identified three distinct monoclonal antibody types: (1) MAbs that bind specifically to human cell lines; (2) MAbs that bind specifically to rodent cell lines; and (3) MAbs that bind to both human and rodent cell lines. Moreover, in some cases there were differences in binding pattern and binding intensity between tumor/transformed and normal cell lines. (These data have been summarized and are presented in the Appendix). This observation is of great interest since the differences may reflect molecules which may be expressed differentially in tumor/transformed and normal cells. Moreover, these molecules could be the ones responsible for the abnormal behavior (aggregation,

compaction, 3-dimensional growth) exhibited by tumor and transformed cells.

The specific aims for the next six-month period are: (1) to continue to expand our MAb library; (2) to continue to characterize the binding pattern and binding intensity of selected MAbs on panels of human and rodent cell lines; and (3) to initiate functional biological assays to determine whether MAbs can modulate the cell-cell interactions (aggregation, compaction) of normal, tumor and transformed cells.

This project also supports the research training of two underrepresented minority students (one graduate and one undergraduate). Both students made significant contributions to the work accomplished during the reporting period. Moreover, each student submitted an abstract and presented a paper at a national scientific meeting (copy included in Appendix). One student was accepted to the University of Texas Medical School at Houston and will start in fall 1994.

Appendix

Tissue Culture Cell Lines

WI38sv40	human sv40 transformed lung fibroblast
WI38	normal human lung fibroblast
Hela $6TG^{r-}$	human cervical carcinoma (6TG resistant)
IMR90sv40	human sv40 transformed lung fibroblast
IMR90	normal human lung fibroblast
GM3320	human neuroblastoma
Hep3b	human hepatoma
2DF*F1	Con A resistant mutant (of B14I50)
B14I50	Chinese hamster peritoneal
CHO 77256	Chinese hamster ovary
FTO-2B	rat liver cells: TK-
Mouse L	mouse fibroblast

Summary of monoclonal antibody **5A12** (WSJ-2) by fluorescence microscopy.

CELL LINE	FLUORESCENCE INTENSITY		
	FIXED	**LIVE**	**PATTERN**
WI38sv40	++	+++	cell-cell
Hela 6TG^{r-}	++	+++	cell-surface
IMR90sv40	++	I.P.	cell-surface with cell-cell
GM3320	++	I.P.	cytoplasmic
Hep3b	+++	I.P.	around edges & cell surface
B14I50	+	I.P.	granular cytoplasmic
2DF*F1	+	+	weak "all over"
CHO 77256	++	I.P.	cytoplasmic
FTO-2B	+++	I.P.	granular cytoplasmic
Mouse L	-	-	

FLUORESCENCE INTENSITY

- = no fluorescence
+ = weak
++ = medium
+++ = strong
++++ = very strong

I.P. = in progress

ANTIBODY 5A12 : This antibody has been renamed WSJ-2 and is a Kappa IgG1 isotype which exhibits a cell- cell binding pattern on WI38sv40 cells.

CELL LINE	FLOW CYTOMETRY *Net Fluorescence
WI38sv40	+++ (333.27)
WI38	++ (236.02)
Hela 6TGr	+ (173.41)
2DF*F1	-
B14I50	-

*NET FLUORESCENCE

-	= < 50
+	= > 100
++	= > 200
+++	= > 300
++++	= > 400

*Net fluorescence values were obtained by subtracting the mean fluorescence value of the negative control, from the mean fluorescence value of the monoclonal antibody being tested. (Mean fluorescence values wer convertd from log to linear prior to calculations (log_{10} X 256)).

Summary of monoclonal antibody **10D8** (WSJ-3) by fluorescence microscopy.

CELL LINE	FLUORESCENCE INTENSITY		
	FIXED	**LIVE**	**PATTERN**
WI38sv40	+++	+++	cell-cell
Hela 6TG^{r-}	++	+++	intermittant
IMR90sv40	+++	I.P.	cell-surface with cell-cell
GM3320	++	I.P.	cytoplasmic
Hep3b	+	I.P.	around edges of cell
B14I50	+	I.P.	cell-surface, some cell-cell
2DF*F1	++	+	cell-surface & between cells
CHO 77256	+++	I.P.	cytoplasmic
Hep3b	+	I.P.	around edges of cell
FTO-2B	+	I.P.	granular cytoplasmic
Mouse L	-	-	

FLUORESCENCE INTENSITY

- = no fluorescence
+ = weak
++ = medium
+++ = strong
++++ = very strong

I.P. = in progress

ANTIBODY 10D8 : This antibody has been renamed WSJ-3 and is a Kappa IgG1 isotype which exhibits a cell- cell binding pattern on WI38sv40 cells.

CELL LINE	FLOW CYTOMETRY *Net Fluorescence
WI38sv40	+++ (308.67)
WI38	++ (210.45)
Hela 6TGr	+ (166.44)
2DF*F1	-
B14I50	-

*NET FLUORESCENCE

-	= < 50
+	= > 100
++	= > 200
+++	= > 300
++++	= > 400

*Net fluorescence values were obtained by subtracting the mean fluorescence value of the negative control, from the mean fluorescence value of the monoclonal antibody being tested. (Mean fluorescence values wer convertd from log to linear prior to calculations (log_{10} X 256)).

Summary of monoclonal antibody **8E10** (WSJ-4) by fluorescence microscopy.

CELL LINE	FLUORESCENCE INTENSITY		
	FIXED	LIVE	PATTERN
WI38sv40	+++	+++	cell-cell
Hela 6TG^{r-}	++	+++	cell - surface
IMR90sv40	++	I.P.	cell-surface with cell-cell
GM3320	++	I.P.	cytoplasmic
Hep3b	+++	I.P.	around edges & cell surface
B14I50	+	I.P.	granular cytoplasmic
2DF*F1	-	-	
CHO 77256	++	I.P.	cytoplasmic
FTO-2B	+++	I.P.	granular cytoplasmic
Mouse L	-	-	

FLUORESCENCE INTENSITY

- = no fluorescence
+ = weak
++ = medium
+++ = strong
++++ = very strong

I.P. = in progress

ANTIBODY 8E10 : This antibody has been renamed WSJ-4 and is a Kappa IgG1 isotype which exhibits a cell-cell binding pattern on WI38sv40 cells.

CELL LINE	FLOW CYTOMETRY *Net Fluorescence	
WI38sv40	+++	(316.99)
WI38	++	(219.70)
Hela 6TGr	+	(165.66)
2DF*F1	-	
B14I50	-	

*NET FLUORESCENCE

-	= < 50
+	= > 100
++	= > 200
+++	= > 300
++++	= > 400

*Net fluorescence values were obtained by subtracting the mean fluorescence value of the negative control, from the mean fluorescence value of the monoclonal antibody being tested. (Mean fluorescence values wer convertd from log to linear prior to calculations (log_{10} X 256)).

Summary of monoclonal antibody **6A3** (WSJ-5) by fluorescence microscopy.

CELL LINE	FLUORESCENCE INTENSITY		
	FIXED	LIVE	PATTERN
WI38sv40	+++	+++	cell-surface
Hela 6TG^{r-}	++	+++	cell-surface
IMR90sv40	+++	I.P.	cell-surface
GM3320	+++	I.P.	granular cell-surface
Hep3b	-	I.P.	
B14I50	-	I.P.	
2DF*F1	-	-	
CHO 77256	-	I.P.	cytoplasmic
FTO-2B	-	I.P.	granular cytoplasmic
Mouse L	-	-	

FLUORESCENCE INTENSITY

- = no fluorescence
+ = weak
++ = medium
+++ = strong
++++ = very strong

I.P. = in progress

ANTIBODY 6A3 : This antibody has been renamed WSJ-5 and is a Kappa IgG1 isotype which exhibits a cell-surface binding pattern on WI38sv40 cells.

CELL LINE	FLOW CYTOMETRY *Net Fluorescence
WI38sv40	+++ (367.87)
WI38	+++ (390.22)
Hela 6TGr	++++ (401.68)
2DF*F1	-
B14I50	-

*NET FLUORESCENCE

-	= < 50
+	= > 100
++	= > 200
+++	= > 300
++++	= > 400

*Net fluorescence values were obtained by subtracting the mean fluorescence value of the negative control, from the mean fluorescence value of the monoclonal antibody being tested. (Mean fluorescence values wer convertd from log to linear prior to calculations (log_{10} X 256)).

Summary of monoclonal antibody **3C3** (WSJ-6) by fluorescence microscopy.

CELL LINE	FLUORESCENCE INTENSITY		
	FIXED	**LIVE**	**PATTERN**
WI38sv40	+++	+++	cell-surface
Hela $6TG^{r-}$	++	+++	cell-surface
IMR90sv40	++++	I.P.	cell-surface
GM3320	-	I.P.	
Hep3b	-	I.P.	
B14I50	-	I.P.	
2DF*F1	-	-	
CHO 77256	-	I.P.	
FTO-2B	-	I.P.	
Mouse L	-	-	

FLUORESCENCE INTENSITY

- = no fluorescence
+ = weak
++ = medium
+++ = strong
++++ = very strong

I.P. = in progress

ANTIBODY 3C3 : This antibody has been renamed WSJ-6 and is a Kappa IgG1 isotype which exhibits a cell-surface binding pattern on WI38sv40 cells.

CELL LINE	FLOW CYTOMETRY *Net Fluorescence
WI38sv40	++++ (413.83)
WI38	++++ (423.81)
Hela 6TGr	++++ (452.11)
2DF*F1	-
B14I50	-

*NET FLUORESCENCE

-	= < 50
+	= > 100
++	= > 200
+++	= > 300
++++	= > 400

*Net fluorescence values were obtained by subtracting the mean fluorescence value of the negative control, from the mean fluorescence value of the monoclonal antibody being tested. (Mean fluorescence values wer convertd from log to linear prior to calculations (log_{10} X 256)).

Summary of monoclonal antibody **DF1.2** (BDF1.2) by fluorescence microscopy.

CELL LINE	FLUORESCENCE INTENSITY		
	FIXED	**LIVE**	**PATTERN**
B14I50	+++	+++	cell-surface & cell-cell
2DF*F1	+++	+++	cell-surface & between cells
CHO 77256	+++	I.P.	cell-surface;some cell to cell
FTO-2B	+	I.P.	weak cell surface
WI38sv40	+	I.P	weak cell surface
Hela 6TG^{r-}	-	I.P.	n/a
IMR90sv40	-	I.P.	n/a
GM3320	+/-	I.P.	binds only a subpopulation of cells; between cells

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FLUORESCENCE INTENSITY

- = no fluorescence
+ = weak
+/- = subpop
++ = medium
+++ = strong
++++ = very strong

I.P. = in progress

ANTIBODY DF1.2 : This antibody has been renamed BDF1.2 and is a Kappa IgG1 isotype which exhibits a cell- cell binding pattern on 2DF*F1 and B14I50 cells.

CELL LINE	FLOW CYTOMETRY *Net Fluorescence
2DF*F1	++++ (524.30)
B14I50	++++ (515.35)
WI38sv40	- (-6.6)
Hela 6TGr	- (-3.65)

*NET FLUORESCENCE

- = < 50
+ = > 50
++ = > 200
+++ = > 300
++++ = > 400

*Net fluorescence values were obtained by subtracting the mean fluorescence value of the negative control, from the mean fluorescence value of the monoclonal antibody being tested. (Mean fluorescence values wer convertd from log to linear prior to calculations (log_{10} X 256)).

Summary of monoclonal antibody **DF4.2** (BDF4.2) by fluorescence microscopy.

CELL LINE	FLUORESCENCE INTENSITY		
	FIXED	**LIVE**	**PATTERN**
B14I50	-	-	n/a
2DF*F1	++	+++	speckled/cell-cell
CHO 77256	+++	I.P.	cell-surface;some cell to cell
FTO-2B	-	I.P.	n/a
WI38sv40	-	I.P	n/a
Hela 6TG^{r-}	-	I.P.	n/a
IMR90sv40	-	I.P.	n/a
GM3320	-	I.P.	n/a

FLUORESCENCE INTENSITY

- = no fluorescence
+ = weak
+/- = subpop
++ = medium
+++ = strong
++++ = very strong

I.P. = in progress

ANTIBODY DF4.2 : This antibody has been renamed BDF4.2 and is a Kappa IgG1 isotype which exhibits a fragmented (speckled) binding pattern including localization to intercellular boundaries and extracellular matrix.

CELL LINE	FLOW CYTOMETRY *Net Fluorescence	
2DF*F1	+	(185.8)
B14I50	-	(2.85)
WI38sv40	-	(-6.6)
Hela 6TGr	-	(-3.65)

*NET FLUORESCENCE

-	= < 50
+	= > 50
++	= > 200
+++	= > 300
++++	= > 400

*Net fluorescence values were obtained by subtracting the mean fluorescence value of the negative control, from the mean fluorescence value of the monoclonal antibody being tested. (Mean fluorescence values wer convertd from log to linear prior to calculations ($\log_{10}$ X 256)).

Summary of monoclonal antibody **10H10** (BDF7.1) by fluorescence microscopy.

CELL LINE	FLUORESCENCE INTENSITY		
	FIXED	**LIVE**	**PATTERN**
B14I50	+ or -	+/-	cell surface; some cell-cell
2DF*F1	+++	+++	speckled/cell-cell
CHO 77256	+++	I.P.	speckled/cell-cell
FTO-2B	-	I.P.	n/a
WI38sv40	-	I.P	n/a
Hela $6TG^{r-}$	-	I.P.	n/a
IMR90sv40	-	I.P.	n/a
GM3320	+/-	I.P.	n/a

FLUORESCENCE INTENSITY

- = no fluorescence
+ = weak
+/- = subpop
++ = medium
+++ = strong
++++ = very strong
+ or - = unstable

I.P. = in progress

ANTIBODY DF10H10 : This antibody has been renamed BDF7.1 and is a Kappa IgG1 isotype which exhibits a fragmented (speckled) binding pattern including localization to intercellular boundaries and extracellular matrix.

CELL LINE	FLOW CYTOMETRY *Net Fluorescence	
2DF*F1	-	(10.05)
B14I50	-	(10.0)
WI38sv40	+	(159.15)
Hela 6TGr	+	(71.75)

*NET FLUORESCENCE

-	= < 50
+	= > 50
++	= > 200
+++	= > 300
++++	= > 400

*Net fluorescence values were obtained by subtracting the mean fluorescence value of the negative control, from the mean fluorescence value of the monoclonal antibody being tested. (Mean fluorescence values wer convertd from log to linear prior to calculations (log_{10} X 256)).

Summary of monoclonal antibody **DF3.2** (BDF3.2) by fluorescence microscopy.

CELL LINE	FLUORESCENCE INTENSITY		
	FIXED	**LIVE**	**PATTERN**
B14I50	-	-	n/a
2DF*F1	+++	+++	speckled/cell-cell
CHO 77256	+++	I.P.	speckled/cell-cell
FTO-2B	+	I.P.	cell surface
WI38sv40	+	I.P	cell surface
Hela 6TG^{r-}	+	I.P.	cell surface
IMR90sv40	+	I.P.	cell surface
GM3320	+	I.P.	cell surface

FLUORESCENCE INTENSITY

- = no fluorescence
+ = weak
+/- = subpop
++ = medium
+++ = strong
++++ = very strong

I.P. = in progress

Summary of monoclonal antibody **2G11** (BDF5.1) by fluorescence microscopy.

CELL LINE	FLUORESCENCE INTENSITY		
	FIXED	**LIVE**	**PATTERN**
B14I50	-	-	n/a
2DF*F1	+++	++++	speckled/cell-cell
CHO 77256	+++	I.P.	speckled/cell surface
FTO-2B	+	I.P.	cell surface
WI38sv40	-	I.P	n/a
Hela 6TG^{r-}	-	I.P.	n/a
IMR90sv40	-	I.P.	n/a
GM3320	-	I.P.	n/a

FLUORESCENCE INTENSITY

- = no fluorescence
+ = weak
+/- = subpop
++ = medium
+++ = strong
++++ = very strong

I.P. = in progress

Summary of monoclonal antibody **9C6** (BDF6.1) by fluorescence microscopy.

CELL LINE	FLUORESCENCE INTENSITY		
	FIXED	**LIVE**	**PATTERN**
B14I50	-	-	n/a
2DF*F1	++	+++	speckled/cell-cell
CHO 77256	+++	I.P.	speckled/cell surface
FTO-2B	+	I.P.	cell surface
WI38sv40	-	I.P	n/a
Hela 6TG^{r-}	-	I.P.	n/a
IMR90sv40	-	I.P.	n/a
GM3320	-	I.P.	n/a

FLUORESCENCE INTENSITY

- = no fluorescence
+ = weak
+/- = subpop
++ = medium
+++ = strong
++++ = very strong

I.P. = in progress

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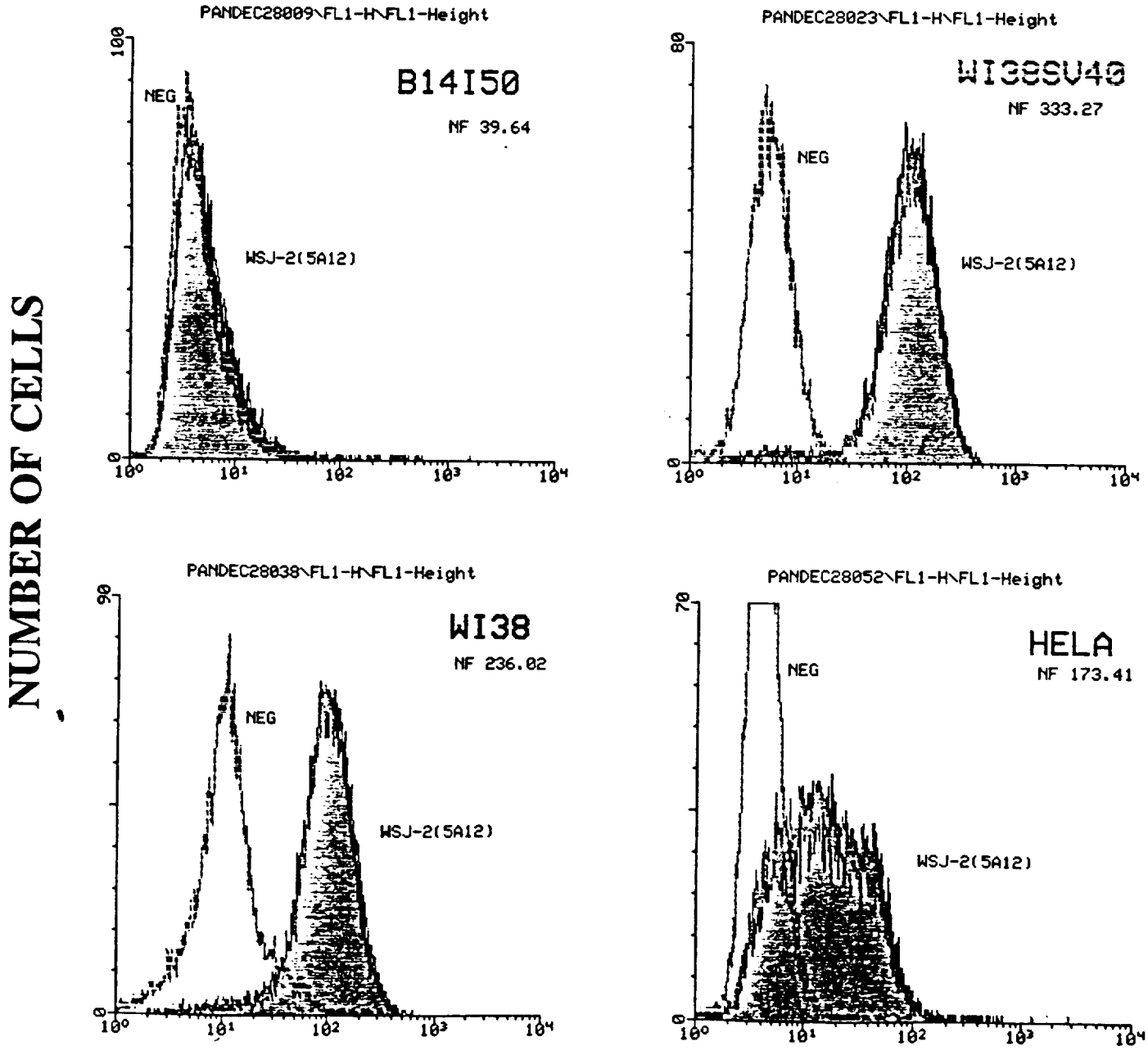

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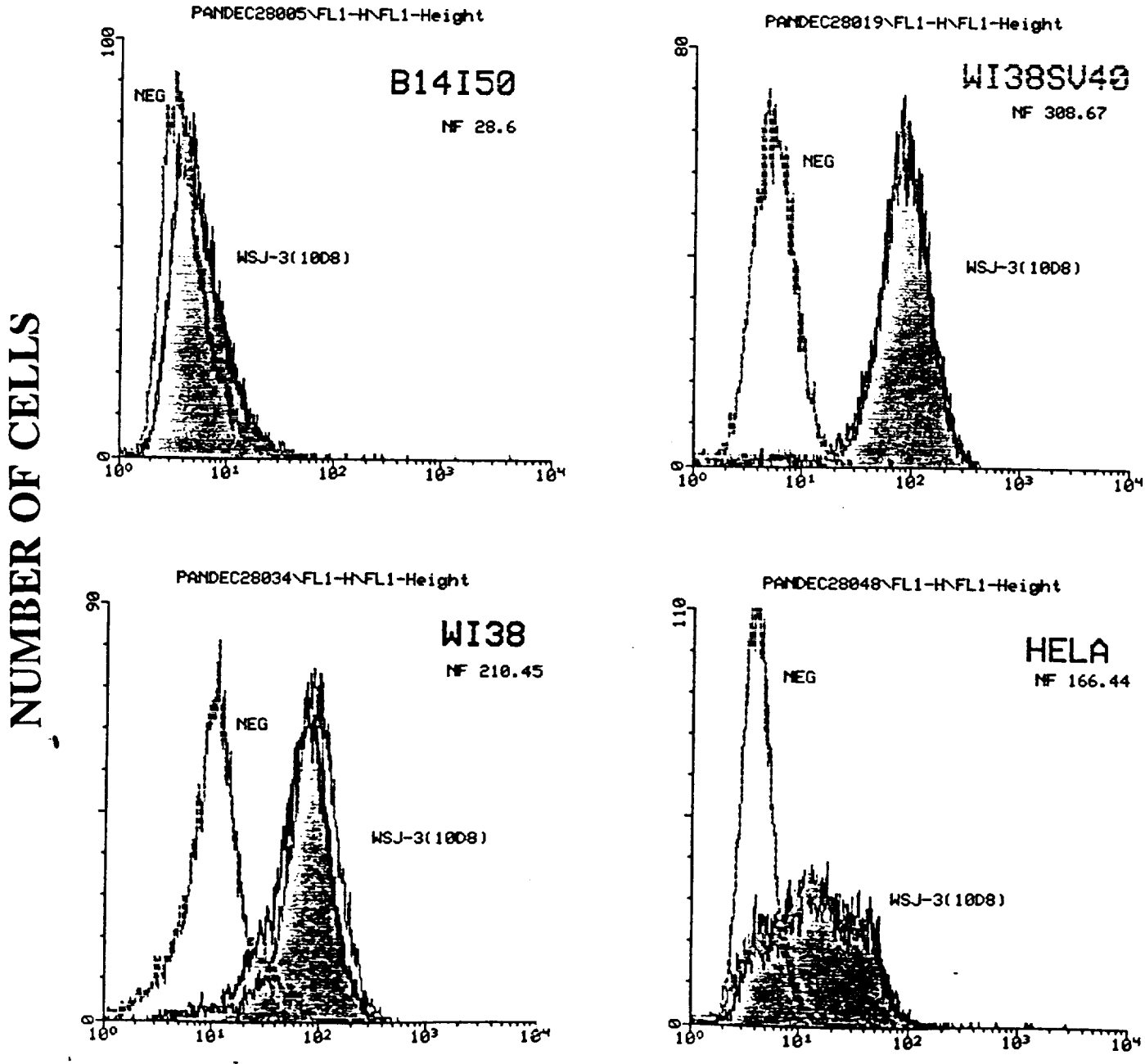

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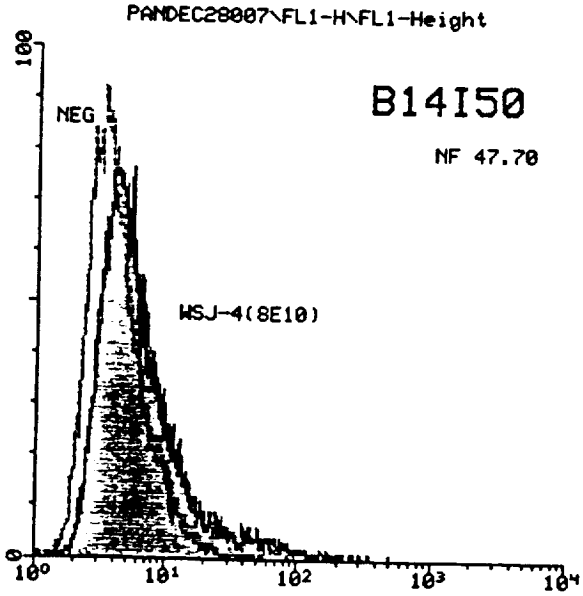

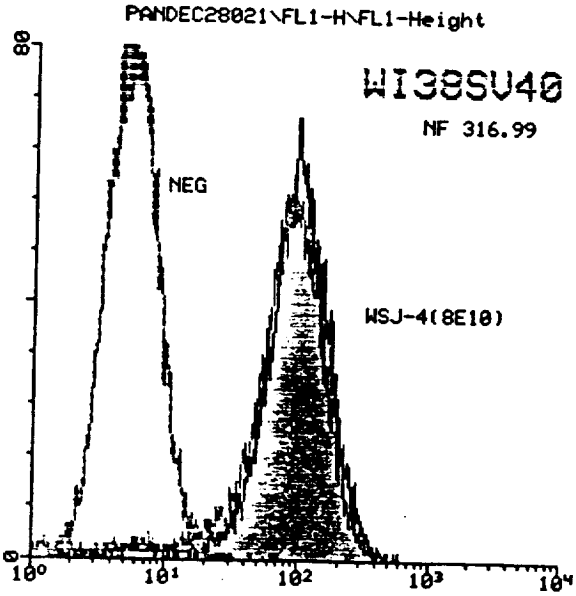

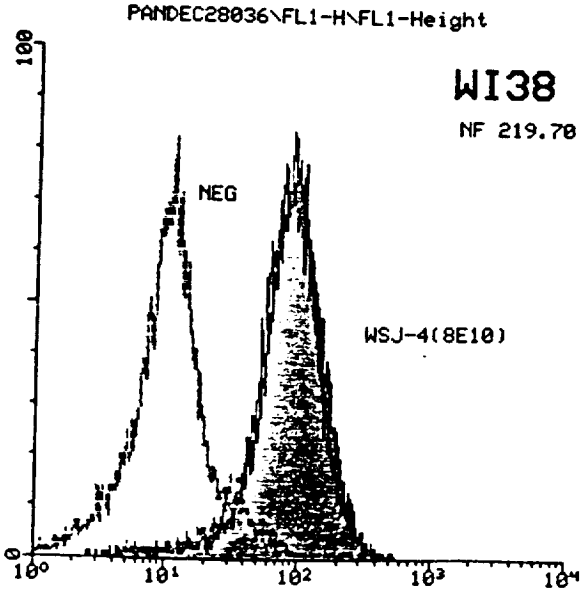

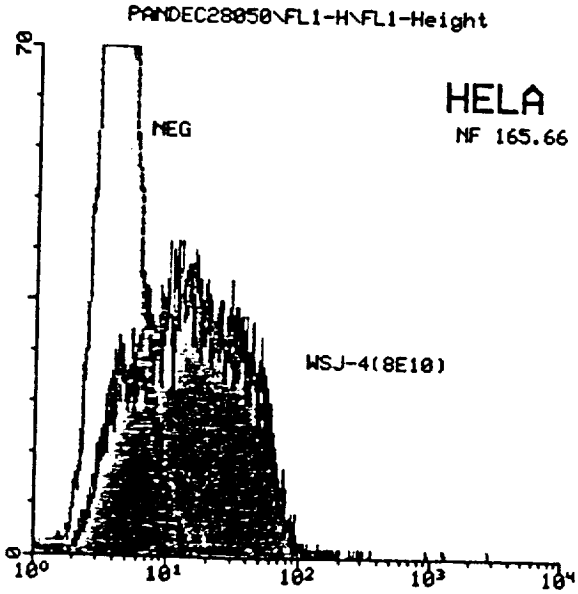

NUMBER OF CELLS

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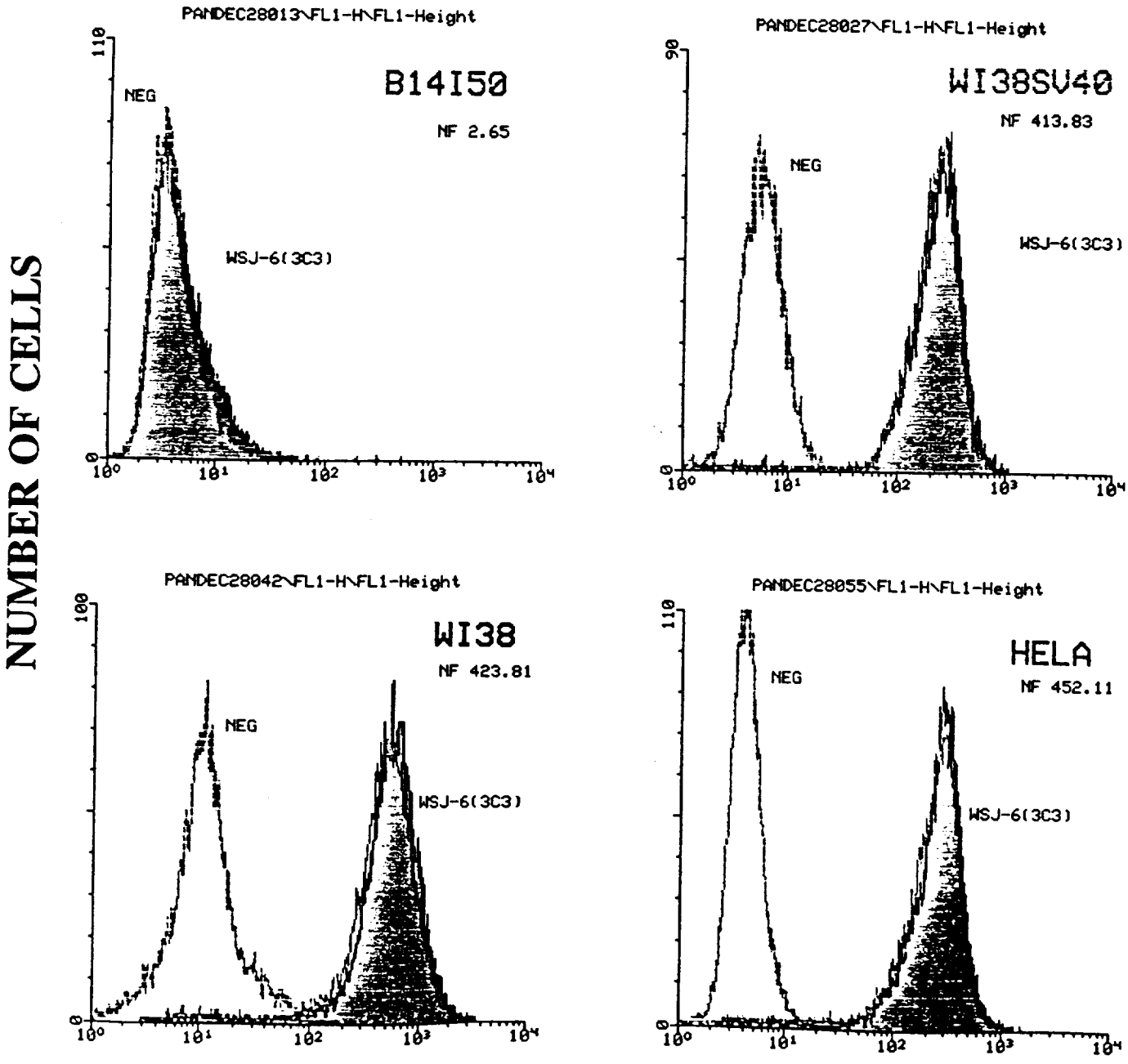

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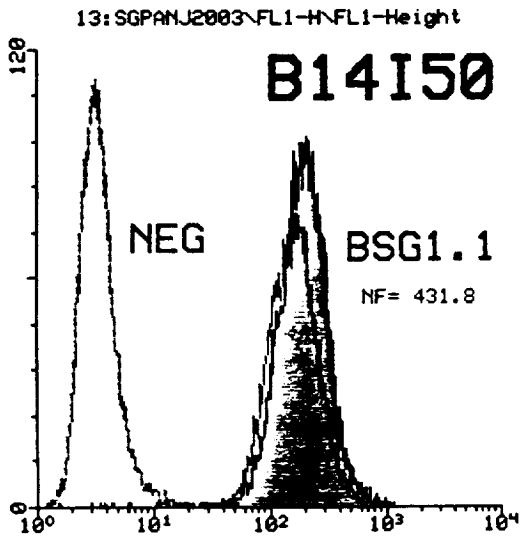

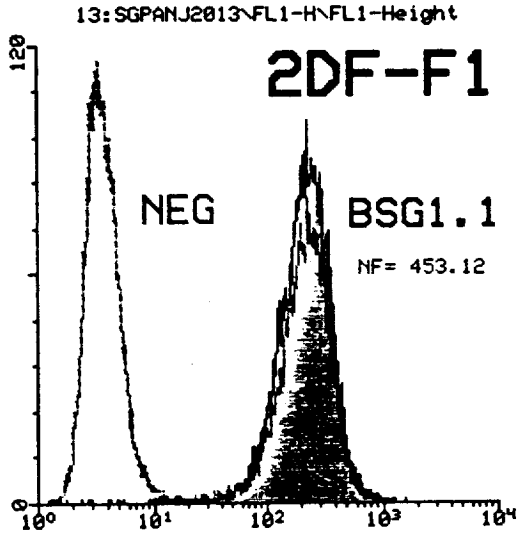

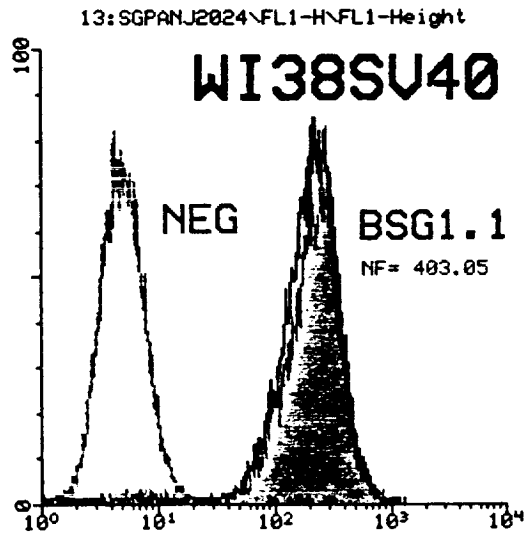

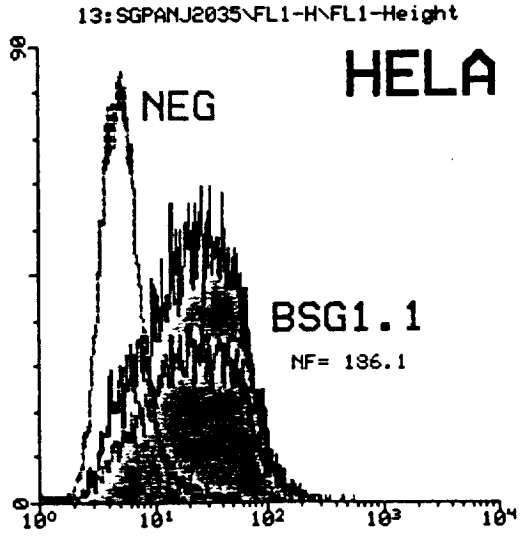

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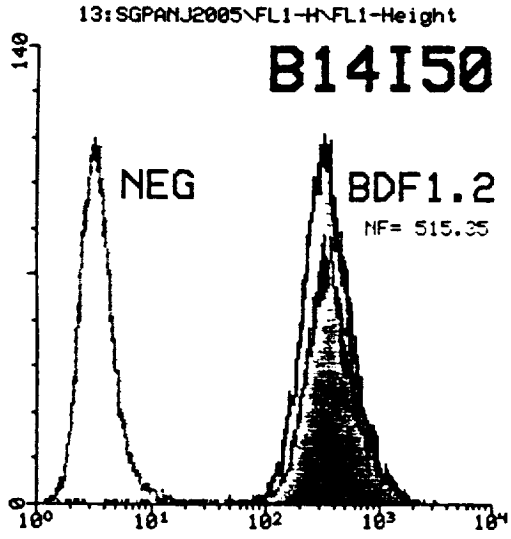

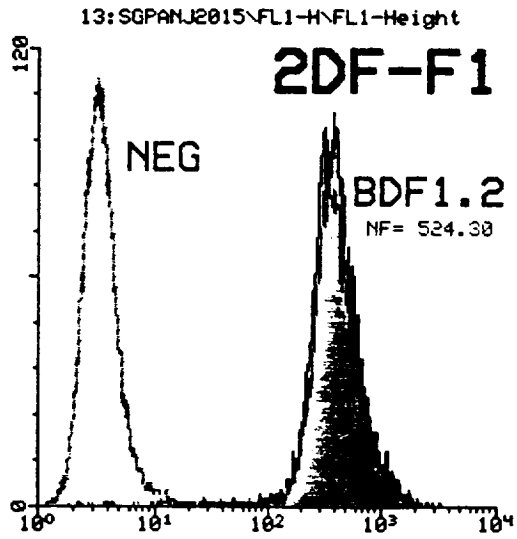

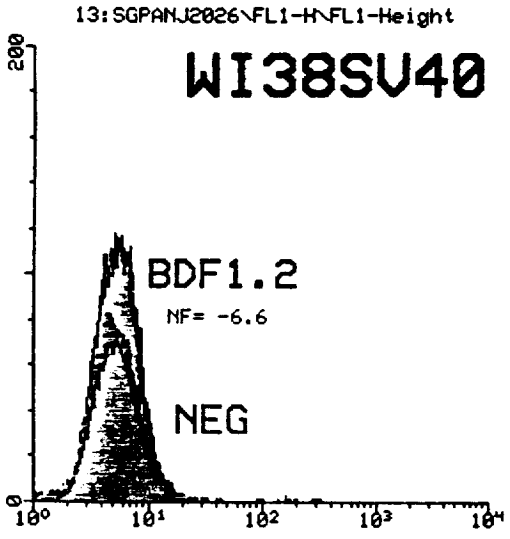

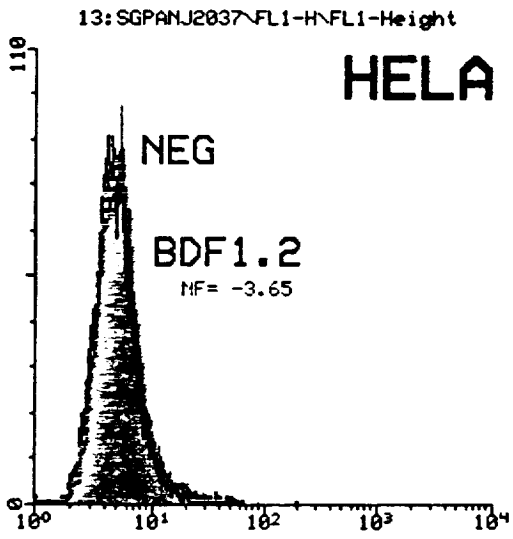

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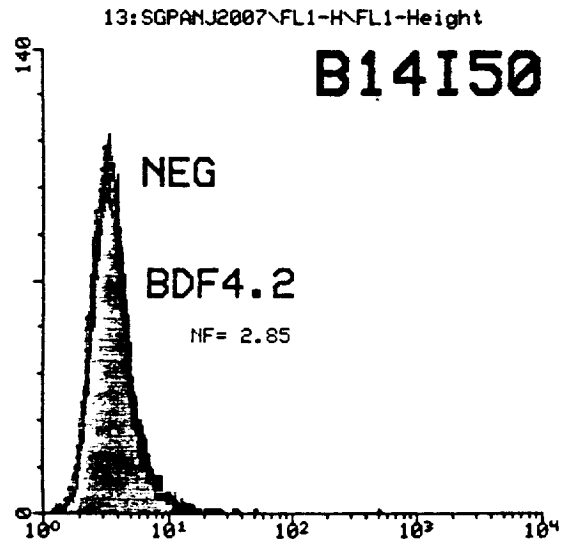

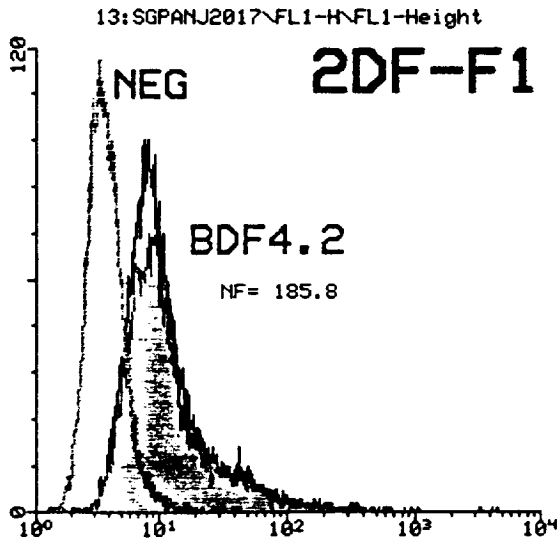

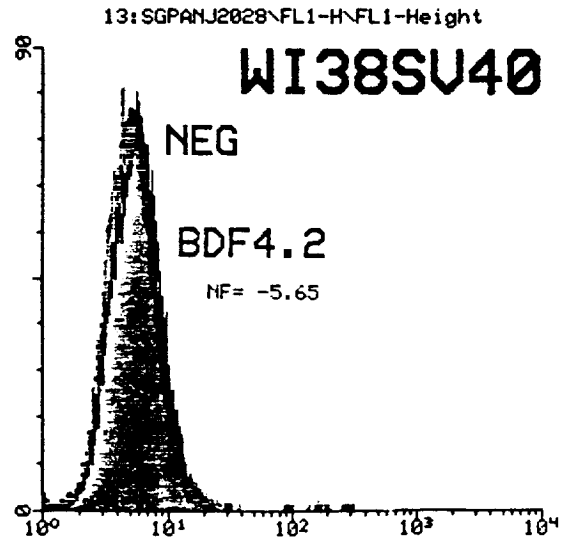

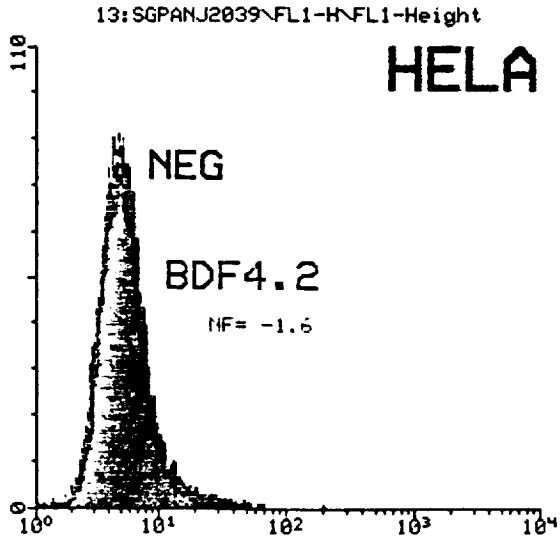

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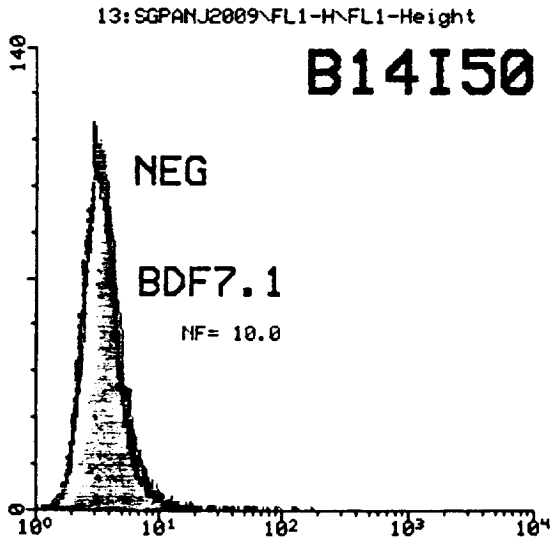

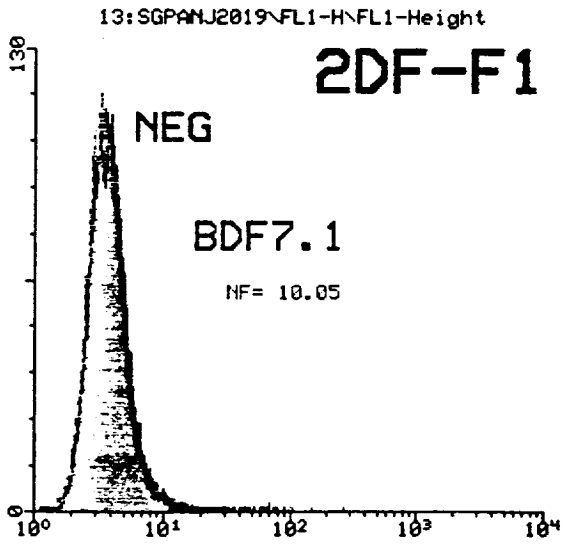

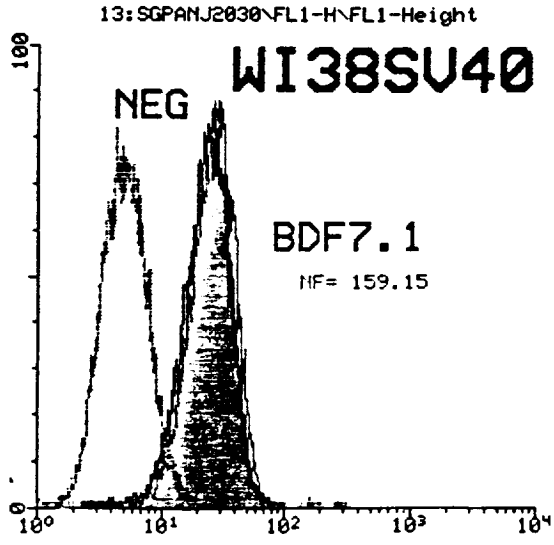

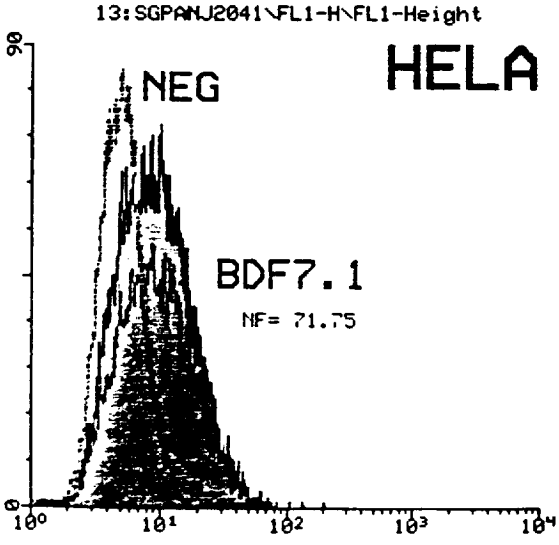

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ISOLATION OF MONOCLONAL ANTIBODIES TO SURFACE MOLECULES OF A COMPACTION MUTANT OF THE MULTICELL SPHEROID PHENOTYPE

We are using multicell spheroids (MCS) to study cell-cell interactions of transformed cells. MCS are formed via cellular processes (i.e., aggregation and compaction) mediated by surface molecules. We have produced several mutants of B14I50 (hamster) cells which are defective in MCS formation. In this study, we have used a subtractive immunization technique to isolate monoclonal antibodies (MAbs) to surface molecules of a compaction-defective mutant, ConA^{R}-2DF-F1. Initial screening by fluorescence microscopy revealed two major types of MAb-surface binding patterns. One type of MAb preferentially localized to intercellular boundaries, while a second type exhibited a more general staining pattern including localization to intercellular boundaries and extracellular matrix. Studies are now in progress to determine whether these MAbs block MCS formation of wildtype B14I50 cells. (Supported by NSF (RII-9005546), NASA (NAG 2-819) and NIH (GM 08194) grants).

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Classification: Undergraduate - Senior
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NSF Program: RIMI
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Presentation Setting: Panel

MONOCLONAL ANTIBODIES TO MULTICELL SPHEROIDS OF HUMAN TRANSFORMED CELLS USING A SUBTRACTIVE IMMUNIZATION TECHNIQUE. CG Cantu, LA Jordan, LS Armstrong, AO Martinez, The University of Texas at San Antonio, Division of Life Sciences, San Antonio, TX 78230.

Multicell tumor spheroids (MTS) are three-dimensional *in vitro* models used in our laboratory to study cellular and molecular mechanisms involved in cell-cell interactions. MTS are formed via a two-step process involving aggregation and compaction mediated by surface molecules of tumor and transformed cells. In this study, we are using a subtractive immunization technique to increase specificity to surface molecules involved in cell-cell interactions. This scheme involved multiple injections of WI38SV40 single cells (tolerogen) each followed by injection of the immunosuppression drug cyclophosphamide. Multiple injections of WI38SV40 spheroids (immunogen) were then given, theoretically generating antibodies that recognize the molecules present on the immunogen that are not present on the tolerogen. This technique has allowed us to isolate an increased number of MAbs that exhibit binding patterns at points of cell-cell contact by florescence microscopy. The MAbs will be further characterized by functional assays and flow cytometry. Future studies will utilize the MAbs to identify surface adhesion molecules involved in MTS formation by Western blot analysis. (Supported by NIH (GM 08194), and NASA (NAG 2-819) grants, The University of Texas at San Antonio)

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